133-27

DRAFT

29 July 1963

MEMORANDUM FOR : THE RECORD

SUBJECT : Continuation of MKULTRA, Subproject #133

1. The purpose of MKULTRA, Subproject 133 is to enable TSD/[redacted] to utilize the services of [redacted] Associate Professor of Biology B at [redacted] B

2. [redacted] continues to provide data and materials which lend themselves to new and unique approaches to energy production and transformation (bio-batteries). His studies on basic mechanisms of mineral transformations also provide new potential paths for deterioration of metals.

3. B The Geschickter Fund for Medical Research will function as covers B and cut-out during this fiscal year. The cost of this program for one year will be $9,000.00 to which must be added $360.00 which represents a 4% service charge to be retained by the cut-out B The total cost of the program will not exceed $9,360.00. Charges should be made against Allotment No. 4125-1390-3902.

4. It is not anticipated that permanent equipment other than that listed in the budget will be required for this program. Title to the equipment listed will be retained by the B Institute in lieu of higher overhead rates.

(b)(3)

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5. Documentation and accounting for travel expenses which are reimbursable by [redacted] will conform to the accepted practice of that organization.

6. [redacted] has been cleared COVERTLY and is unwitting and will remain unwitting of the true nature of the sponsor.

[redacted]
TSD/[redacted] Branch

[redacted]
Chief
TSD/[redacted] Branch

[redacted]
Chief, Sci. TSD/[redacted]

Attachment:
Proposal

Distribution:
Original only

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Signed Copy

[redacted]

Proposal No. [redacted]

entitled

[redacted]

for renewal of Grant-in-aid B

for the period

1 September 1963 - 31 August 1964

Submitted on behalf of

[redacted] C/B

Associate Professor of Biology

April 1963

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PURPOSE OF STUDY

This proposal is a request for renewal for the period 1 September 1963-31 August 1964 of the grant-in-aid presently supporting research on [redacted]. The field of investigation covered by the present grant is so broad and relatively unexplored that several additional years of research can be profitably spent in unraveling the problem. The results of the research carry increasing significance not alone academically but also practically, as in public health, conservation of mineral resources, mineral exploration, and mineral extraction. In public health, these investigations are providing clues to the possible origin and control of acid ferruginous, and/or acid cupriferous, and/or acid arsenical stream pollution, deriving from bacterial action on natural mineral deposits in certain geographical locations[1]. With continued population increase, maintenance of all water resources in a condition fit for human consumption becomes more and more important. The work is also providing additional clues to the understanding of manganese biochemistry in public water supplies, where manganese together with iron can cause discoloration of water and water containers, and where manganese and iron are deposited biologically in water mains and pipes. In the area of conservation of mineral resources, the work under this grant provides a basis for assessing and controlling any relative instability of mineral deposits in relation to microbes. It seems evident that uncontrolled bacterial leaching could conceivably lead to depletion and eventual complete translocation of some deposits. In mineral exploration, this work indicates that special groups of microorganisms known to be able to live at the expense of a specific mineral may be of great assistance in discovering new sites of mineral deposits. Finally, this work lends additional support to the practice of using microbes in mineral extraction.

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SUMMARY OF WORK ACCOMPLISHED UNDER THE 1962-63 GRANT FROM THE GESCHICKTER FUND

(a) Microbial Action on Mineral Sulfides

Quantitative studies on the action of the Thiobacillus-Ferrobacillus group of bacteria on the minerals orpiment (As_2S_3), arsenopyrite (FeS_2FeAs_2), and enargite ($3Cu_2SAs_2S_5$), were initiated. The bacteria achieved significant solubilization of orpiment, releasing arsenic as arsenite and arsenate. Oxidation of the sulfur portion of orpiment is probable, but remains to be experimentally verified. Some spontaneous, nonbiological oxidation of orpiment occurred, but it was only about one-third as extensive as with bacteria. The chemistry of bacterial oxidation of orpiment appears to differ significantly from nonbiological oxidation as reflected by pH changes during the processes. With bacteria the pH fell from 3.5 to 2.0 in thirty-five days, but without bacteria it rose from 3.5 to 5.0 in that time. The precise chemical mechanism of orpiment oxidation remains to be worked out.

Quantitative work on bacterial oxidation of arsenopyrite showed release of iron, arsenic, and probably sulfur, from the mineral. Most of the analyses in this study were carried out by an undergraduate student for his senior thesis. The results have shown that, contrary to a sustained release of soluble arsenic from orpiment by bacteria, only a limited amount of soluble arsenic was released by them from arsenopyrite. This happened in spite of a pronounced release of iron. The reason for limited release of soluble arsenic by bacteria from arsenopyrite is the precipitation of iron arsenites and arsenates after a critical concentration of soluble iron and arsenite and arsenate has been reached. Although some oxidation of arsenopyrite occurred in the absence of bacteria, its extent was less and its chemistry different, because iron arsenates and arsenites were not precipitated without bacteria, and the pH of the medium dropped from 3.5 to 2.5 in thirty-six days with bacteria and rose from 3.5 to 4.0 in that time without bacteria.

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The extensive reprecipitation of iron and arsenic through bacterial action has direct implications with respect to possible translocation of the constituents of arsenopyrites in nature. The bacterial phenomenon also poses a problem when arsenic and iron are to be extracted together from a natural mineral deposit.

Quantitative work on bacterial oxidation of enargite has given results resembling those with arsenopyrite. Although the bacteria are evidently acting on the mineral, a sustained solubilization of the component arsenic is not noted. Indeed, in the presence of bacteria the dissolved arsenic concentration drops after an initial rise. In the absence of bacteria the arsenic concentration rises slightly but continually. It is not clear from the results so far what the fate of the arsenic or copper is after bacterial action because no recognizable precipitate was formed. Contaminating iron is released extensively by bacteria from the mineral but not without them. The released iron appears to remain in the ferrous state. The pH changes are from 3.5 to 2.5 with bacteria, and from 3.5 to 4.5 without bacteria, in thirty-six days. A precise description of the chemical changes that enargite undergoes remains to be worked out.

Growth on cuprous sulfide by the Thiobacillus-Ferrobacillus group, investigated chiefly by a graduate student, is showing variable responses on different synthetic preparations. Bacterial enrichment cultures derived from several mine effluents during the past year have, however, given more consistent growth responses on these preparations. The possibility arises that consistent action on cuprous sulfide requires the participation of more than one organism. In this connection, the principal investigator found protozoa and fungi accompanying the Thiobacillus-Ferrobacillus group of bacteria in mine water from Inspiration, Arizona. The protozoa included an amoeba and a flagellate, which appeared to grow at the expense of the bacteria and fungi. The amoeba were observed to ingest such organisms.

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Both kinds of protozoa are unusual in their tolerance of 800 ppm copper and upwards of 2000 ppm iron. Ordinarily these metal concentrations would be expected to be lethal. The flagellate has been repeatedly subcultured in an iron-salts medium in mixed culture with the Thiobacillus-Ferrobacillus group of bacteria.

(b) Manganese Nodules

During three cruises in the Atlantic Ocean in June, July, and August, 1962, as guest of the [redacted] thirty-four manganiferous samples and twenty-four cores of bottom deposits were taken. About two-thirds of the manganiferous samples have now been tested for their bacterial content and for the ability of these bacteria in aiding manganese addition to the respective samples, as previously described by [redacted] Most of these samples were also analyzed for total iron and manganese content. The results showed that the various manganiferous samples were by no means alike. In some cases this was obvious by visual inspection. In other cases, however, the differences were not macroscopically apparent. Instead, the iron-manganese content, or the bacterial content, or the Mn adsorptive power of the samples, showed differences. Results of experiments, designed to test for the enhancement of Mn adsorption by manganiferous material with the help of the native bacterial flora, could be divided into four major categories. In one, bacterial enhancement of Mn adsorption occurred with or without prior surface-sterilization. In a second, it occurred only with surface sterilization, and in a third only without it. In a fourth, bacterial enhancement of Mn adsorption was not noted under either condition. Since not all bacteria from Mn nodules can enhance Mn adsorption, inclusion of surface-sterilization in the procedure causes selection of different types of organisms from the nodule flora than omission of surface-sterilization. However, the full explanation for the variability of bacterial enhancement of Mn adsorption is more complicated than that. With a number of

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samples it was noted that Mn uptake by manganiferous material resulted in a net loss of manganese from the adsorber. The reaction accounting for this phenomenon is the result of a dismutation between Mn^{2+} and MnO_2 and will be discussed further below. This reaction was exhibited by about one-sixth of the samples tested and shows that the properties of these samples are different from the rest. It can therefore be concluded that chemical and physical properties of manganiferous materials help to determine the effectiveness of bacteria in enhancing Mn adsorption. Moreover, since all but one of the samples of the 1962 collection came from extinct submarine volcanoes (sea mounts), and since quite a few of these samples behaved differently from earlier samples from other sources, it is postulated that geographic location of manganiferous samples may affect their chemical, physical, and biological properties.

A clue to the nature of nonbiological Mn release from manganiferous samples is provided by an experiment which shows a dismutation reaction between Mn^{2+} and MnO_2 forming Mn^{3+} when nodules adsorb Mn^{2+}, on the condition that Mn^{3+} is stabilized so as to prevent it from undergoing the reverse reaction. Pyrophosphate was found to be an effective stabilizer. Since pyrophosphate does not occur in a marine environment, protein or amino acids may serve instead as stabilizers. Nodule breakdown can thus be accomplished biologically with sugar as the reducing agent of MnO_2,[2] or nonbiologically by a dismutation reaction between Mn^{2+} and MnO_2. Why the latter reaction occurs with only some nodules, even though all have MnO_2 and adsorb Mn^{2+}, remains to be explained.

The ability of Arthrobacter to aid in Mn addition to nodular substance was shown in pure culture experiments. From similar tests it could be concluded that Vibrio can similarly aid in Mn addition, but not Achromobacter. All three organisms have been found in nodules. Arthrobacter, at high peptone concentrations, causes clumping and heaping of nodular material; not so at low peptone concentrations. A similar phenomenon has been noted in soil . The clumping and heaping

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seems to reduce surface area, and therefore slows adsorption of Mn.

Initial experiments have revealed that Arthrobacter can aid in Mn adsorption by "synthetic" MnO_2. However, the adsorption and incorporation process of Mn^{2+} is not permanent. The previously cited dismutation reaction seems to come into play eventually, probably when the bacteria become physiologically inactive at the end of their growth cycle. "Synthetic" MnO_2 is a much poorer adsorbent than nodule material. It is postulated that the iron in nodule material helps to stabilize the manganese in it and, for as yet unknown reasons, increases its adsorptive power.

The core samples of bottom deposits, collected last summer, were examined by enrichment for bacterial content. The distribution of bacteria in these cores was not uniform, nor were the bacteria necessarily of the same kind at different depths in the same core. These findings are similar to those cited by Zo Bell[4]. Work is presently under way to test the ability of the core samples to adsorb Mn with and without bacterial growth. Differences in Mn adsorptive capacity seem to exist among cores on the basis of tests so far. Such differences, if corroborated by further tests, must reflect upon Mn distribution in the sea and upon nodule distribution and structure.

REFERENCES

1. de Grys, Ann. Econ. Geol. 57: 1031-1044 (1962).

[redacted]

3. Martin, J.P., and S.A. Waksman. Soil Science 50: 29-47 (1940).

4. Zo Bell, C.E. MARINE MICROBIOLOGY. Chronica Botanica Co., Publ. 1946, p. 91.

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PROGRAM OF FUTURE WORK

(a) Mineral Sulfides

Although it is the intention to continue testing other mineral sulfides for susceptibility to attack by the Thiobacillus-Ferrobacillus group, special attention will be paid to the details of bacterial action on orpiment, arsenopyrite, and enargite. Quantitative methods have to be developed for determining the proportions of various forms of a given element dissolved and reprecipitated after bacterial action. Such information should provide clues to the mechanism of action by the bacteria. The detailed information on the fate of arsenopyrite and enargite, when available, will provide information concerning the effect of side reactions on the overall process of bacterial mineral oxidation. Ultimately, it is hoped to develop methods of mass culture of a desired organism on a particular mineral to get enough cell material for resting cell studies and enzymatic investigations.

(b) Manganese Nodules

Two aims will be followed experimentally in this work. One is the further elucidation of environmental relationships to the process of nodule development or degradation. The second is the elucidation of the biochemical mechanism whereby Mn is added to, or released from, manganese nodules by bacteria. To attain the first aim, samples from various known sources will be studied in respect to physical, chemical, and biological properties, and attempts will be made to correlate the results with geographic location. In studying the manganese fixing capacity of bottom muds, possible correlation with nodule development will be sought. To attain the second aim, further studies with purified cultures from nodules will be made on nodule material and on synthetic MnO_2 to compare and contrast behavior. Since previous experiments have already shown a difference in action by bacteria between nodule material and MnO_2, a condition is provided to compare the effect of iron on the two systems. It is planned to develop more specific assay procedures for

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differentiating the various oxidation states of Mn and thereby to follow the exact fate of adsorbed or desorbed Mn, and to establish more clearly the role bacteria play in this process. The ultimate goal is to study the bacterial action on manganese on an enzymic level.

PUBLICATIONS ARISING OUT OF THE WORK UNDER THIS GRANT FOR 1962-63

(1) [redacted] (Submitted to J. Bacteriol.)

(2) [redacted] (In preparation for submission to Econ. Geol.)

(3) [redacted] Abstract. Bacteriol. Proc., 1963, p. 161.

(4) [redacted] (Invited paper to be published in the Proceedings of the Rudolf's Research Conference, Rutgers University, 5-7 June 1963).

(5) It is anticipated that a paper will be written for submission to Appl. Microbiol. on the work with manganese nodules during the last year.

PERSONNEL FOR 1963-64

Principal Investigator: [redacted] Associate Professor of Biology

Technician: [redacted]

Graduate Student: [redacted] (not presently supported)

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155-a/

April 1963

Proposal No. [redacted]
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[redacted]
for renewal of Grant-in-aid
for the period
1 September 1963 - 31 August 1964

(b)(3)

COST ESTIMATE

Personnel		
Principal Investigator		
1/8 time-academic year	$1,175	
1/4 time-summer months	785	$1,960
Technician-full time		4,330
Total Personnel Payments		$6,290
Consumable Supplies		
Chemicals	$ 400	
Glassware	200	600
Permanent Equipment		520
Travel and Communication		
To scientific meetings	$ 250	
Telephone	35	285
Publication of Reports		200
Total Direct Cost		$7,895
Indirect Cost		
14% of Total Direct Cost		1,105
TOTAL		$9,000

[redacted]

[redacted]

Research Division

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